

Title	THE TRIALS IN THE CYTOLOGICAL DIAGNOSIS OF GASTRIC CANCER PART IV ACID PHOSPHATASE REACTION FOR THE IDENTIFICATION OF MALIGNANT GASTRIC CELLS
Author(s)	YOSHIDA, YOSHIYUKI
Citation	日本外科宝函 (1962), 31(3): 315-332
Issue Date	1962-05-01
URL	http://hdl.handle.net/2433/205446
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

THE TRIALS IN THE CYTOLOGICAL DIAGNOSIS OF GASTRIC CANCER

PART IV ACID PHOSPHATASE REACTION FOR THE IDENTIFICATION OF MALIGNANT GASTRIC CELLS

by

YOSHIYUKI YOSHIDA

From the 2nd Surgical Division, Kyoto University Medical School

(Director : Prof. Dr. YASUMASA AOYAGI)

Received for publication Mar. 10, 1962

INTRODUCTION

In a previous paper (YOSHIDA¹⁾), several kinds of histochemical reactions on gastric cytological specimens were studied, and acid phosphatase reaction by the improved GOMORI's^{2,3)} PbS method was found to be useful to distinguish malignant gastric cells.

However, this reaction has yet many unsolved problems (NEWMAN⁴⁾, PEARSE⁵⁾ and LISON⁶⁾) including unknown influences of many factors which are to be investigated further.

The following is the study about the influences of several factors on acid phosphatase activity in identifying malignant gastric cells.

CHAPTER I

STUDY ON SURGICAL MATERIALS MATERIALS AND METHODS

Stomaches were resected under the diagnosis of gastric cancer or peptic ulcer and subjected primarily to the GOMORI's PbS method. The gastrectomy specimens were collected immediately after removal, placed into a bath of ice-cold acetone for 12 hours at a temperature of 0°~4°C, washed with xylol at room temperature and placed in low-melting paraffin (m. p. 46°~48°C) at the temperature of 52°C. Then, serial sections of 6 μ were cut, deparaffinized and incubated in the following medium at 37°C.

The incubating medium were freshly prepared for each experiment and consisted of : 20 cc of 2 per cent solution of lead nitrate, 40 cc of 3 per cent solution of sodium-beta-glycerophosphate, 120 cc of distilled water and 20 cc of buffer solution. The kind of buffer solution and pH of the medium was various on each experiment. The distilled water used were all demineralized using the ion exchange resin filter. The incubating mixtures were adjusted to the respective pH using a glass electrode.

After the incubation, the sections were removed, washed with distilled water for 6 to 8 times, placed into a 1 per cent solution of ammonium sulfide for 2 minutes. They were mounted in balsam without counterstain.

1) *First experiment :*

In order to examine the effect of incubation time, sections were incubated in the medium of pH 4.6 adjusted with 0.2 M acetate buffer solution for 1, 3, 6, 8, 12 or 24 hours.

II) *Second experiment :*

In order to study the influence of the pH of the medium on the enzyme activity, sections were incubated in the mediums adjusted to pH from 2.3 to 9.4 with the following three kinds of buffer solutions: 0.1 M glycol-HCl buffer solution for pH 2.3~3.0, 0.2 M acetate buffer solution for pH 3.6~5.4 and 0.1 M barbital buffer solution for pH 6.4~9.4. Incubation time was 8 hours.

III) *Third experiment :*

The influence of a series of enzyme inhibitors and activators such as fluoride, tartaric acid, magnesium, calcium and zinc on the enzyme activity was studied. Incubation time was 8 hours and pH of the medium was 3.6.

i) Sections were immersed for one hour in 0.01 M sodium fluoride and incubated by the GOMORI's method in the medium containing sodium fluoride in 0.01 M.

ii) Sections were incubated by the GOMORI's method in the medium containing tartaric acid in 4 per cent.

iii) Sections were immersed for 5 minutes in 0.01 M EDTA (ethylen diamine tetraacetic acid-tetrasodium salt) and processed by the GOMORI's method.

iv) In order to study the genuine effect of the metals, the sections were pretreated for five minutes with 0.01 M EDTA to remove metals originally contained in the tissue. The sections were then washed with distilled water for 6 to 8 times and incubated by the GOMORI's method in the mediums containing calcium chloride, magnesium chloride or zinc acetate in 0.01 M.

RESULTS

I) *First experiment :*

The activity of acid phosphatase in a given incubation time was various according to the kinds of cells.

After one hour incubation cancer cells and inflammatory cells showed a trace of activity, but other cells none. It took 3 hours for the cancer cells and inflammatory cells to show the marked activity, 6 or 8 hours for normal cells of the gastric mucosa and more period of time for the vascular endothelium. After 6 or 8 hours of incubation, in most of cancer cells, the activity was just enough to present the cellular and particularly nuclear structures, while in some, after 8 hours of incubation, the nucleus stained

Fig. 1 : Control section for the first experiment. Human pleomorphic adenocarcinoma of the stomach. 6 μ thick. Hematoxylin and eosin stain. $\times 200$.

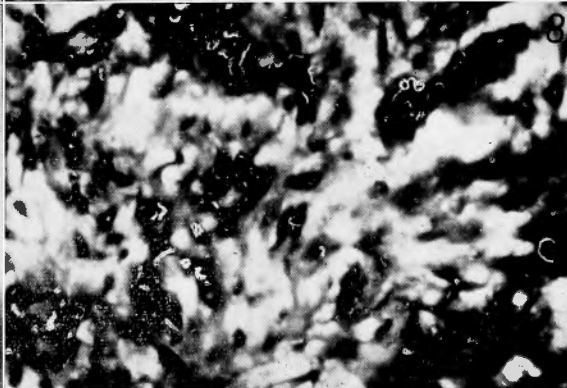
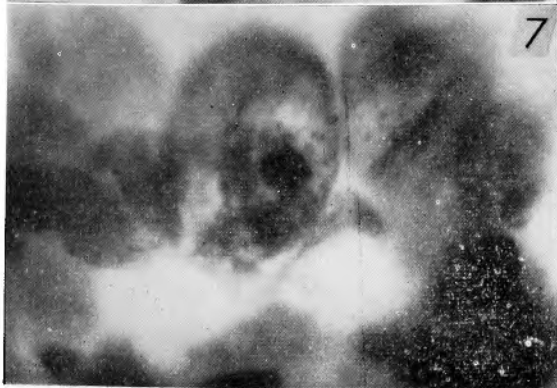
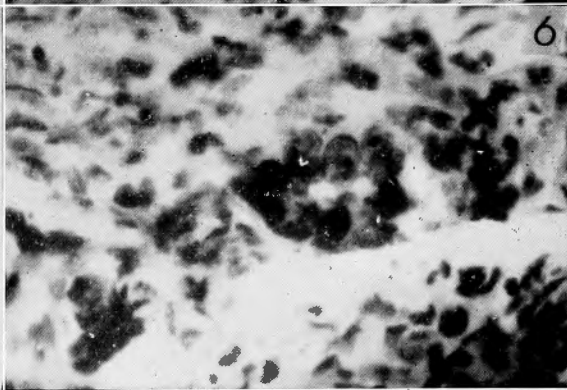
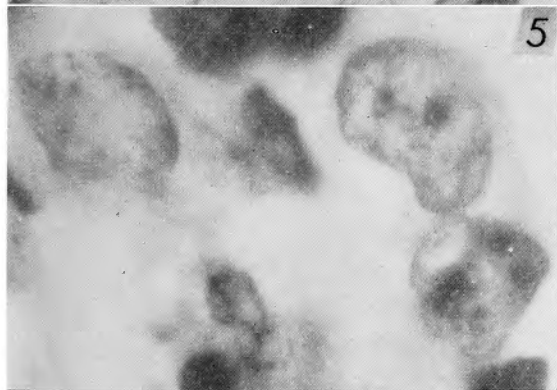
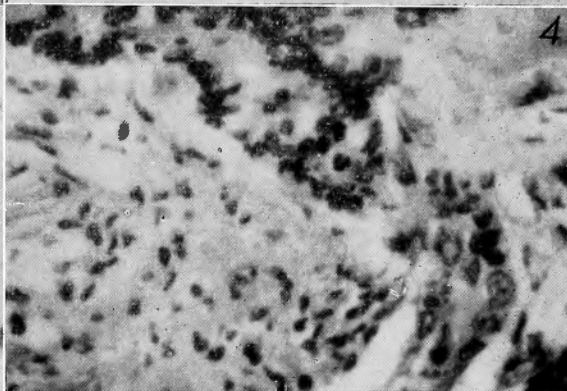
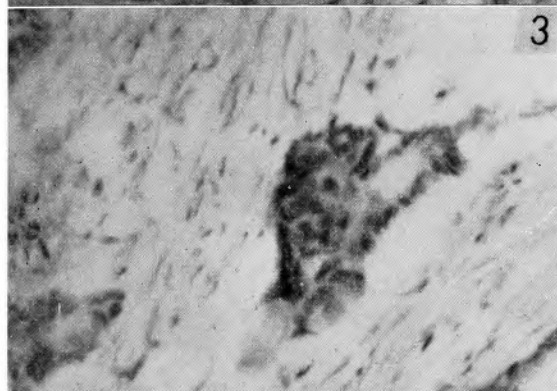
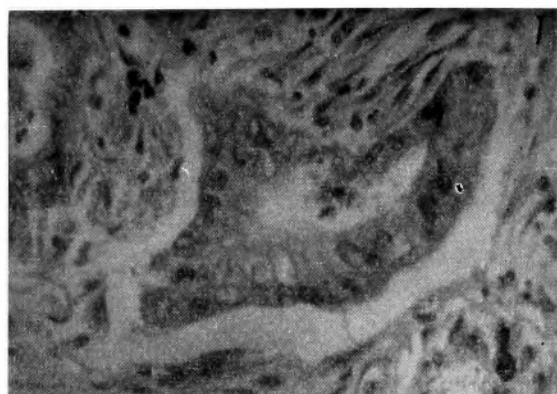
Fig. 2 : The improved Gomori's PbS method with 1 hour incubation in the medium of pH 4.6. The acid phosphatase activity is very light both in tumor cells and inflammatory cells and is hardly noted in other cells. $\times 200$.

Fig. 3 : After 3 hour incubation. The acid phosphatase activity is marked in inflammatory cells and carcinoma cells. $\times 200$.

Fig. 4 and 5 : After 6 hour incubation. Cancer cells showing the marked acid phosphatase activity presenting fine nuclear structures. $\times 200$ and $\times 1000$.

Fig. 6 and 7 : After 8 hour incubation. Cancer cells showing the prominent acid phosphatase activity in the nucleus presenting fine nuclear structures. $\times 200$ and $\times 1000$.

Fig. 8 : After 14 hour incubation. The moderate activity in fibrous stromal cells and the extracellular diffusion of activity is noted. $\times 200$.



in a block revealing no fine cellular structures. The optimal activity of fibrous stromal cells was obtained after 14 hours of incubation, but the muscle fibers and connective tissue showed only a slight activity after 24 hours. The cancer cells and inflammatory cells showed the prominent staining of whole cell with the remarkable extra-cellular diffusion after more than 14 hours of incubation.

As reported in the previous paper, the localization of activity in the nuclei was a common finding except for normal cells of the gastric mucosa which often showed moderate activities in the cytoplasm. If incubated long, however, the cytoplasm of less activity showed feeble staining.

The development of enzyme activity demonstrated in the cells during incubation was as follows. The nuclear membrane and probable nucleolei or reticulum structure were recognized as fine structures in a short incubation time. Other portion of the nucleus showed gradual and uniform increase in the activity. With the increase of incubation time a diffuse staining of the whole cell including the nucleus resulted.

II) Second experiment:

It was found that the grade or the distribution pattern of the enzyme activity and the morphological features of the cells in the stomach cancer tissues varied with the change of the medium pH.

i) The variation of enzyme activity with the change of medium pH was different according to the type of the cell.

Cancer cells showed the most marked activity from pH 3.6 to pH 3.8 with another less prominent peak from pH 7.0 to pH 7.6 in all ranges of medium pH. The similar pattern was observed in normal cells of the gastric mucosa, leucocytes, fibrous stromal cells and muscle fibers. However, the staining from pH 3.6 to pH 3.8 was the most pronounced in cancer cells among other cells. The vascular endothelium showed the strong activity at pH 2.3 and between pH 6.4 and 8.2 with the highest peak at pH 7.0. Erythrocytes had the strongest activity at pH 4.6 and 8.8 and fibrous stromal cells at pH 6.4.

ii) Each cell showed the different grade of enzyme activity at a given pH value. From pH 3.6 to pH 3.8, cancer cells showed the strongest activity followed by inflam-

Fig. 9 : Control section for the second and third experiment. Human primary adenocarcinoma of the stomach. 6 μ thick. Hematoxylin and eosin stain. $\times 200$.

Fig. 10 : The improved GOMORI's PbS method with 8 hour incubation in the medium of pH 2.3. The phosphatase activity is marked in the vascular endothelium, light in cancer cells and none in other cells. The moderate deformation of cellular architecture is noted. $\times 200$.

Fig. 11 : The medium pH is 3.0. Cancer cells and inflammatory cells showing the light phosphatase activity. $\times 200$.

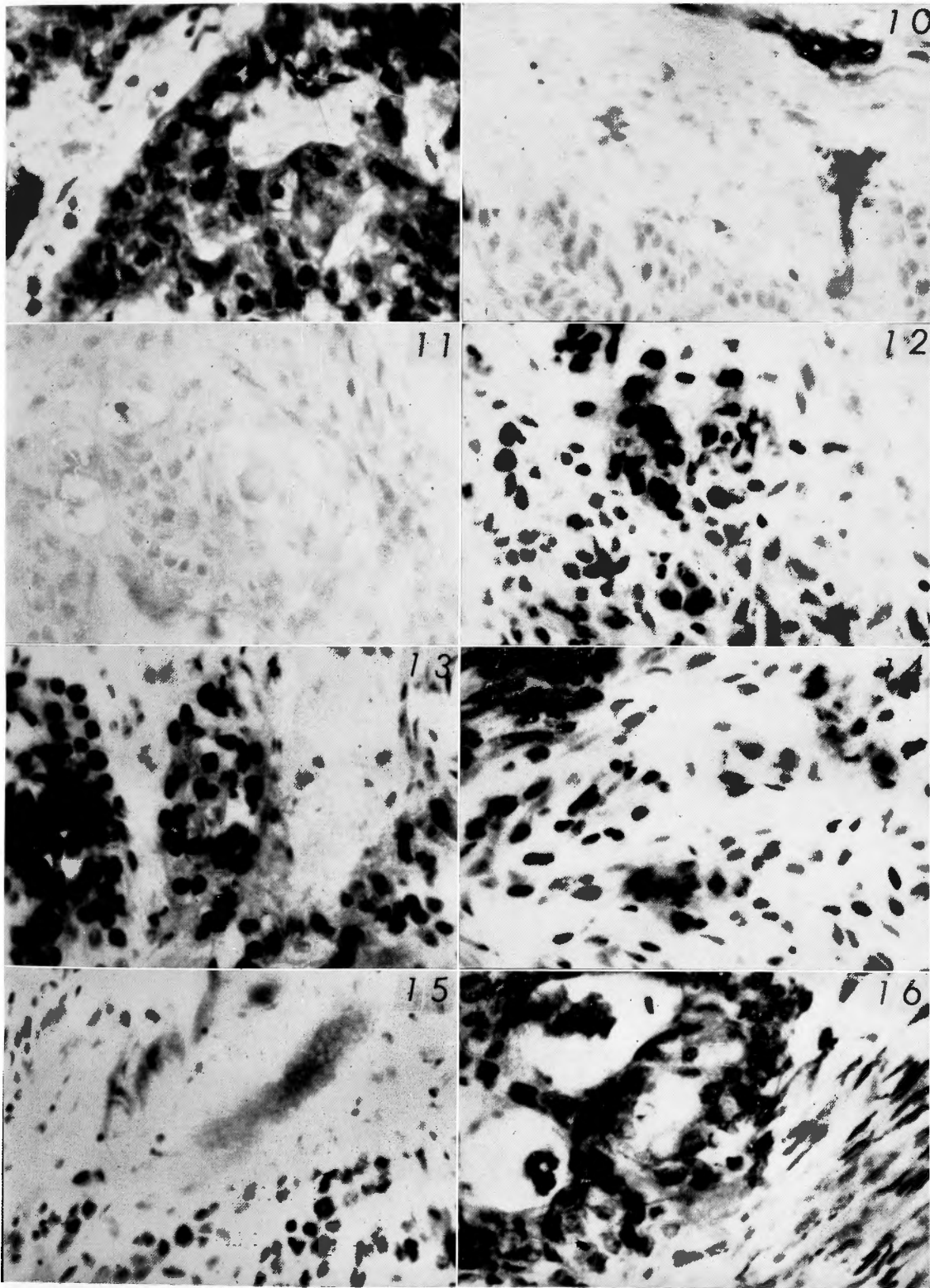
Fig. 12 : The medium pH is 3.6. Cancer cells showing the strongest activity followed by inflammatory cells. The cellular and nuclear structures are well preserved almost without deformation or shrinkage of cells. $\times 200$.

Fig. 13 : The medium pH is 3.8. Cancer cells showing the strongest activity among other cells. $\times 200$.

Fig. 14 : The medium pH is 4.2. Inflammatory cells showing more marked activity than cancer cells. The cellular and nuclear structures are well preserved. $\times 200$.

Fig. 15 : The medium pH is 4.6. Erythrocytes showing the moderate phosphatase activity. $\times 200$.

Fig. 16 : The medium pH is 5.0. The phosphatase activity is light to moderate in the connective tissue. $\times 200$.



matory cells, normal cells of the gastric mucosa, the vascular endothelium and erythrocytes. On the other hand, at this pH range, fibrous stromal cells and muscle fibers showed very light reaction. At pH 4.6, inflammatory cells showed the strongest activity followed by cancer cells and erythrocytes. At pH 6.4 or 7.0, the vascular endothelium showed the strongest activity and inflammatory cells, cancer cells, normal cells of the gastric mucosa and fibrous stromal cells followed in this order.

iii) Cellular and nuclear structure were various according to the respective medium pH

Between pH 3.6 and 5.0 cellular and nuclear structure were fairly well preserved with almost no deformation or shrinkage of cells. Especially the finest cellular and nuclear structure was obtained from pH 3.6 to pH 3.8. Moderate deformation of cellular architecture was noted over pH 5.0. Diffuse and vague cellular architecture was moderately noted between pH 5.4 and 7.6 and below pH 3.0.

iv) Intracellular localization of the activity was various depending on the respective medium pH.

Below pH 3.0 nuclear staining became less with more cytoplasmic staining. Between pH 3.6 and 5.0 strong nuclear and very slight cytoplasmic localization was present. Over pH 5.4 the cytoplasmic staining was increased with the elevation of the medium pH, and over pH 8.2 histiocytes with strong cytoplasmic activity were found scattered in the tissue.

The staining became very weak in the alkaline site over pH 8.2, but the result obtained by the PbS technique was very similar to the findings obtained by the GOMORI's alkaline phosphatase reaction.

III) *Third experiment:*

Sections pretreated with 0.01 M EDTA for 5 minutes showed no enzyme activity in 8 hour incubation and required 24 hours of incubation to obtain as strong staining as shown by the non-pretreated tissue sections after 1 hour incubation.

When the sections pretreated with 0.01 M EDTA for 5 minutes were incubated in the medium contained calcium chloride or magnesium chloride in 0.01 M, the reactions were strongly presented. Especially so was this in the nucleus, demonstrating cellular and nuclear structure clearly. Acid phosphatase activity in cancer cells was more remar-

Fig.17: The medium pH is 5.4. The cellular membrane and the vessel wall showing the marked phosphatase activity. The diffuse and vague cellular architecture is moderately noted. $\times 200$.

Fig.18: The medium pH is 6.4. The deformation of cellular architecture is prominent. $\times 200$.

Fig.19: The medium pH is 7.0. The vessel wall showing the strongest phosphatase activity among others. $\times 200$.

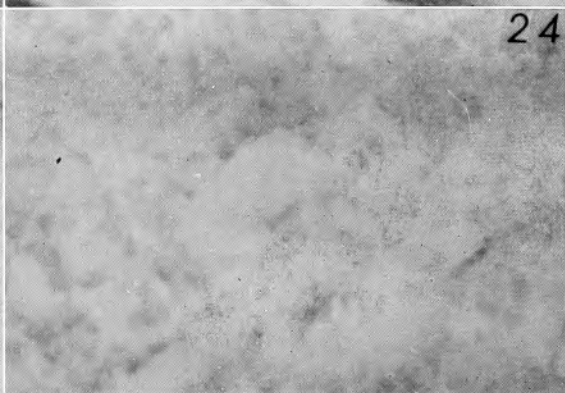
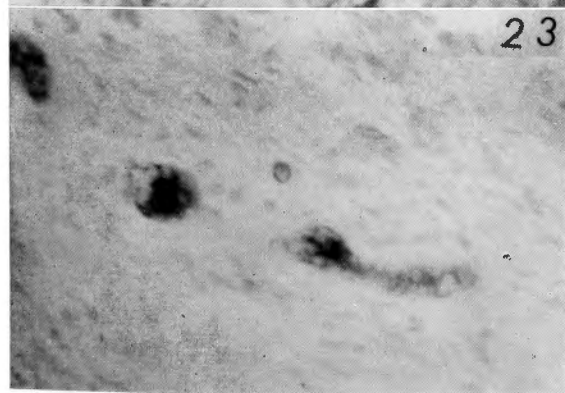
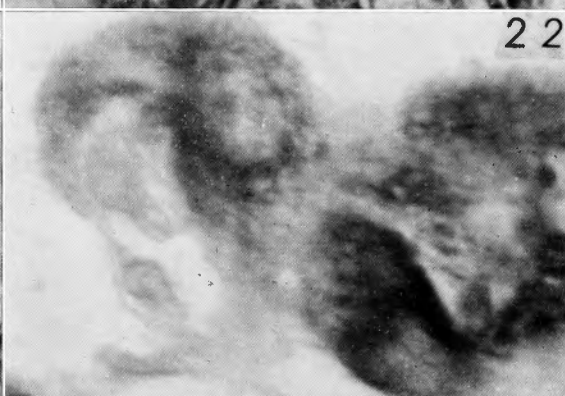
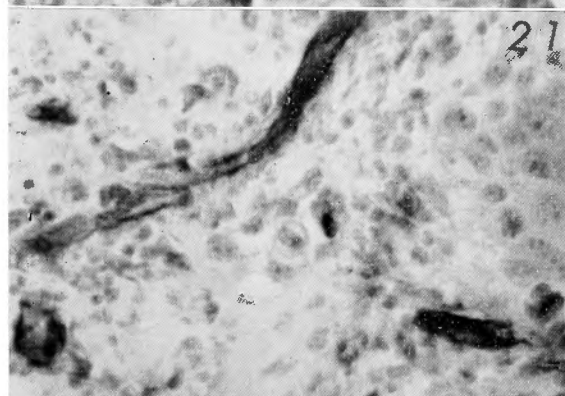
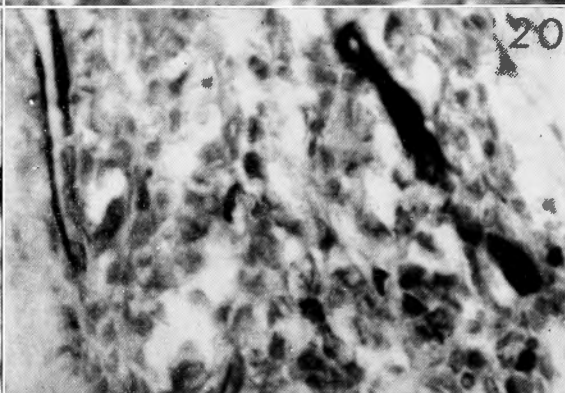
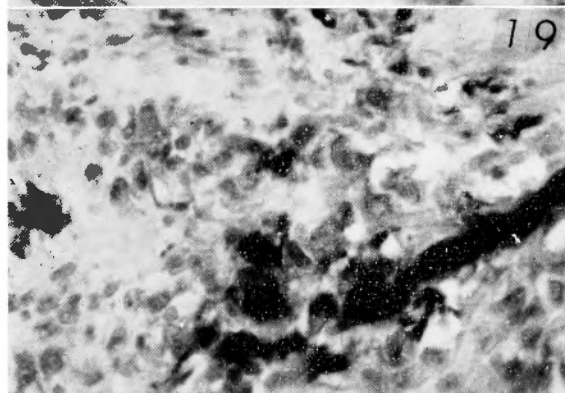
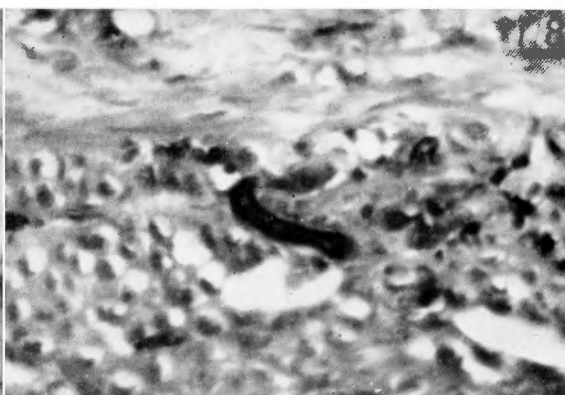
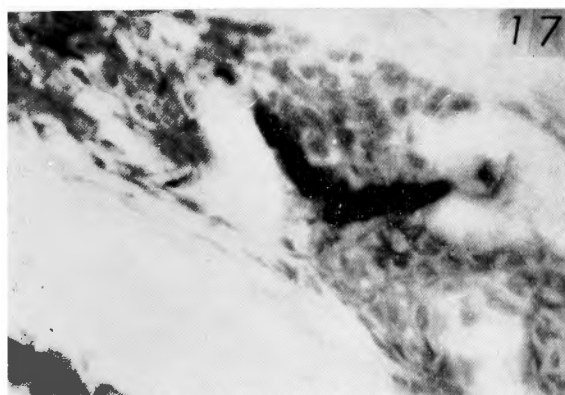
Fig.20: The medium pH is 7.6. Cancer cells showing the moderate phosphatase activity. The diffuse, vague and deformed cellular architecture is noted. $\times 200$.

Fig.21: The medium pH is 8.2. The phosphatase activity is strong in the vessel wall and light in cancer cells and inflammatory cells. $\times 200$.

Fig.22: The medium pH is 8.2. Histiocytes scattered in the tissue with the strong phosphatase activity in the cytoplasm but none in the nucleus. $\times 1000$.

Fig.23: The medium pH is 8.8. The phosphatase activity is moderate in erythrocytes, very light in cancer cells and inflammatory cells, and none in other cells. $\times 200$.

Fig.24: The medium pH is 9.4. The phosphatase activity of all cells is very light with the vague cellular architecture. $\times 200$.



kably presented with calcium ion than with magnesium ion, and if the section was incubated in the medium added with calcium ion, cancer cells showed the strongest activity of the enzyme among other cells. When the section, on the other hand, was incubated in the medium added with magnesium ion, inflammatory cells showed the strongest activity among others followed by cancer cells.

When the section pretreated with EDTA was incubated in the medium added with zink ion, the enzyme activity was not observed.

By treating the section with 0.01 M sodium fluoride or 4 per cent tartaric acid, the enzyme activity was completely absent.

Hitherto, the author used the word "cancer cells" without any regard to the patho-histological type, but the difference of activity among cancer cells in that sense was hardly observed. However, the activity of cancer cells in the infiltrating or proliferating area was more remarkable than that in the other area.

To conclude, the ideal stain method of acid phosphatase activity for the identification of malignant gastric cells is as follows. Specimens pretreated with 0.01 M EDTA for 5 minutes are processed according principally to the GOMORI's PbS method for acid phosphatase reaction, with 8 hour incubation at 37°C in the medium of pH range from 3.6 to 3.8 containing calcium chloride in 0.01 M and glycerophosphate as substrate.

CHAPTER II

STUDY ON CYTOLOGICAL SPECIMENS

MATERIALS AND METHODS

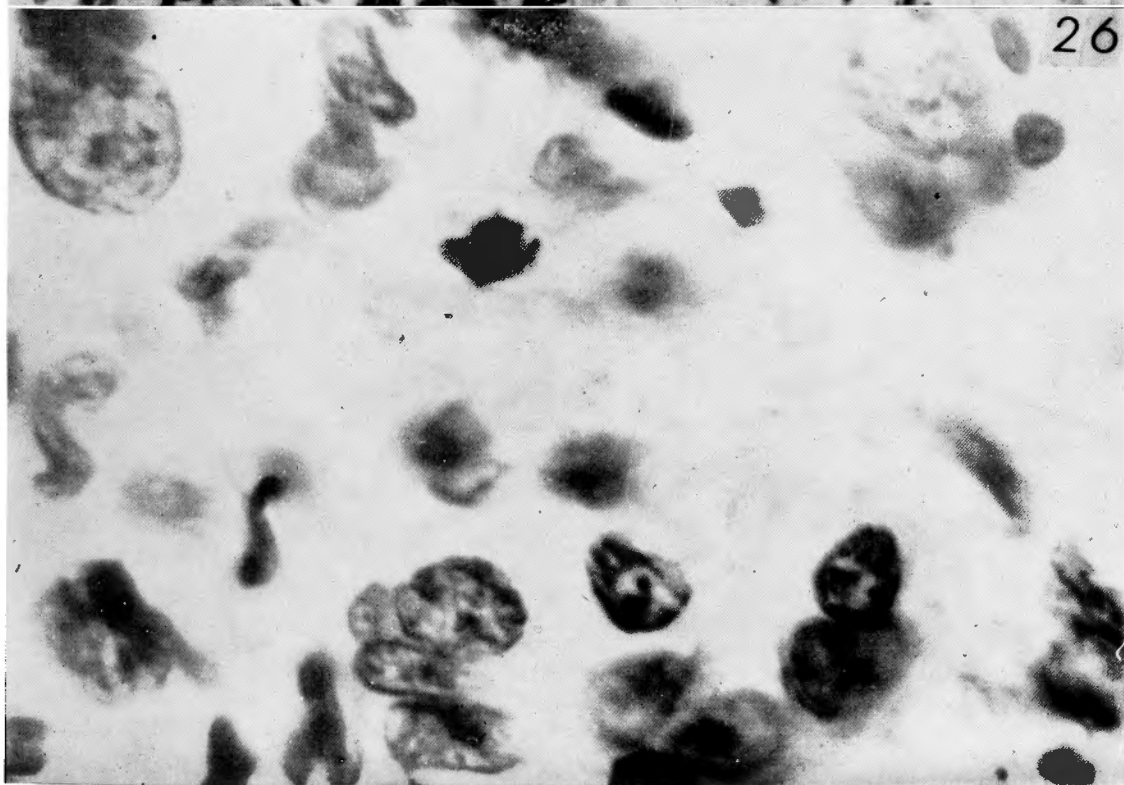
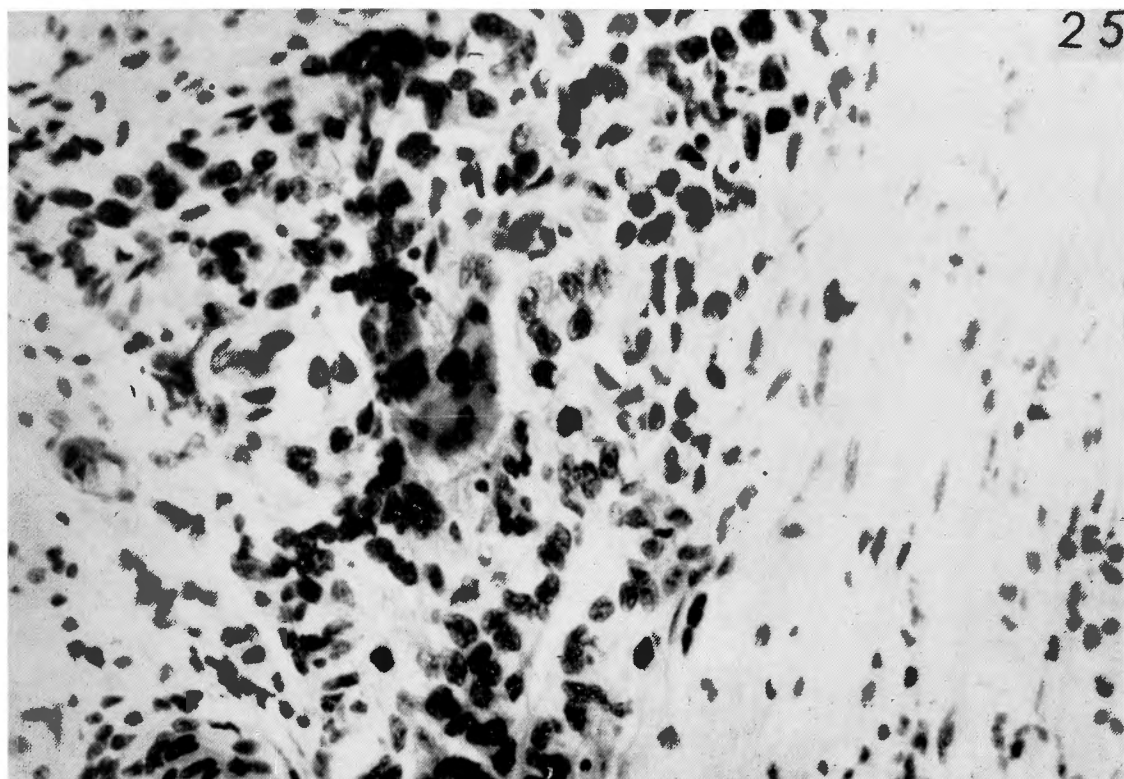
Six patients with gastric disease were subjected to the exfoliative cytology and the balloon pinch smears were obtained by "abrasive balloon with a sheath". Reactions for acid phosphatase on the smear specimens fixed immediately in cold acetone for 12 hours were carried out according to the formula previously mentioned in Chapter I.

The fixed specimens were then washed with distilled water, pretreated with 0.01 M EDTA for 5 minutes and processed according principally to the GOMORI's PbS method with 8 hour incubation at 37°C in the medium of pH range from 3.6 to 3.8 containing calcium chloride in 0.01 M and glycerophosphate as substrate. After the incubation the specimens were washed with distilled water for 6 to 8 times, immersed in 1 per cent solution of ammonium sulfide for 2 minutes and then mounted in balsam without counterstain.

RESULTS

The acid phosphatase activity was observed moderate to marked in most of cancer cells and especially remarkable in those forming a clump, but none in some cancer cells. Inflammatory cells showed very rarely the moderate to marked activity, while normal surface epithelium of the stomach, squamous epithelial cells from the oroesophageal tract and bacilli showed none, except for a few of them presenting a very slight reaction.

Fig.25 and 26 : The section pretreated with 0.01 M EDTA for 5 minutes and processed principally with GOMORI's PbS method by 8 hour incubation in the medium of pH 3.6 containing calcium chloride in 0.01 M. Cancer cells showing the strongest activity with fine cellular and nuclear structures. The activity is marked in the nucleus and light in the cytoplasm. $\times 200$ and $\times 1000$.



Occasionally, the marked activity was found diffuse in the mucous per se of the cytologic specimen from the patient with gastric cancer, whereby cancer and inflammatory cells scattered in the mucous also showed the prominent activity. Intracellular localization of the enzyme activity was mainly in the nucleus, except for cancer cells forming a clump and cells scattered in the mucous, where it was found remarkably in the cytoplasm.

Nuclei of some cancer cells were stained markedly and uniformly without presenting the fine structure, while others were stained slight to marked presenting fairly fine structure. A few of cancer cells showed markedly stained area probably of the nucleolei.

Out of the 6 patients examined cytologically 4 were proved to have malignant gastric lesions by surgery, and cytologic specimens stained with this method presented malignant cells in 3 cases and non-malignant cells in 1 case.

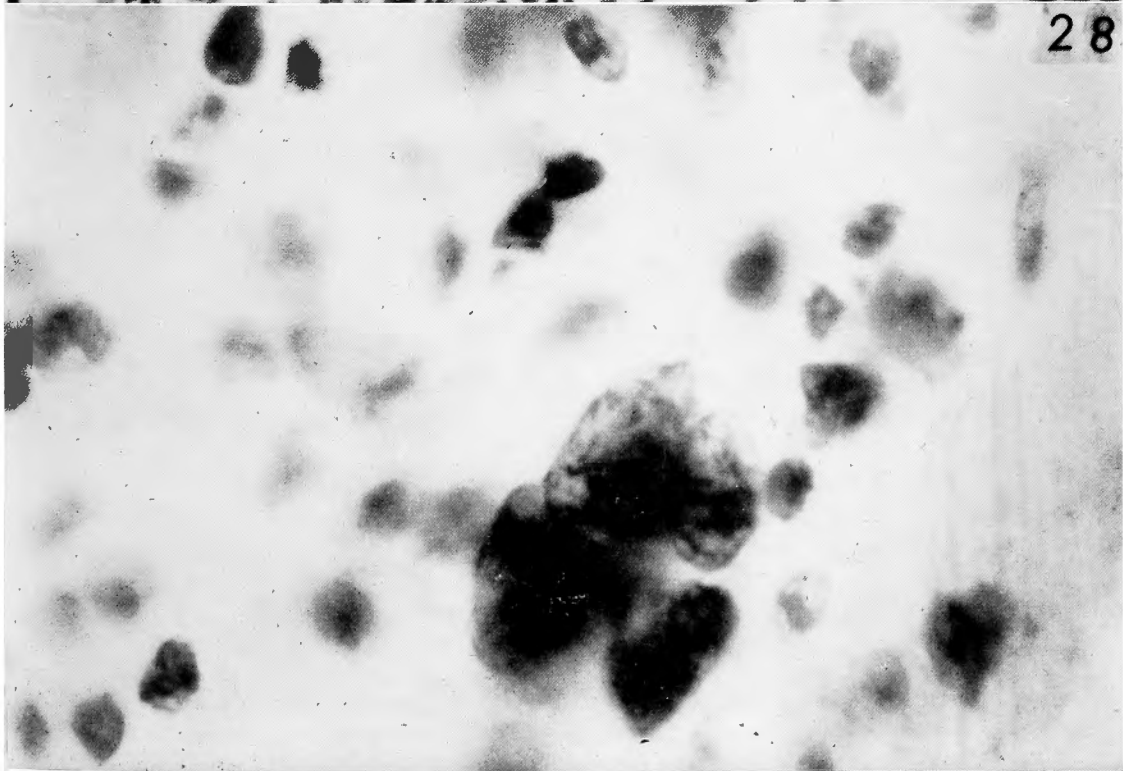
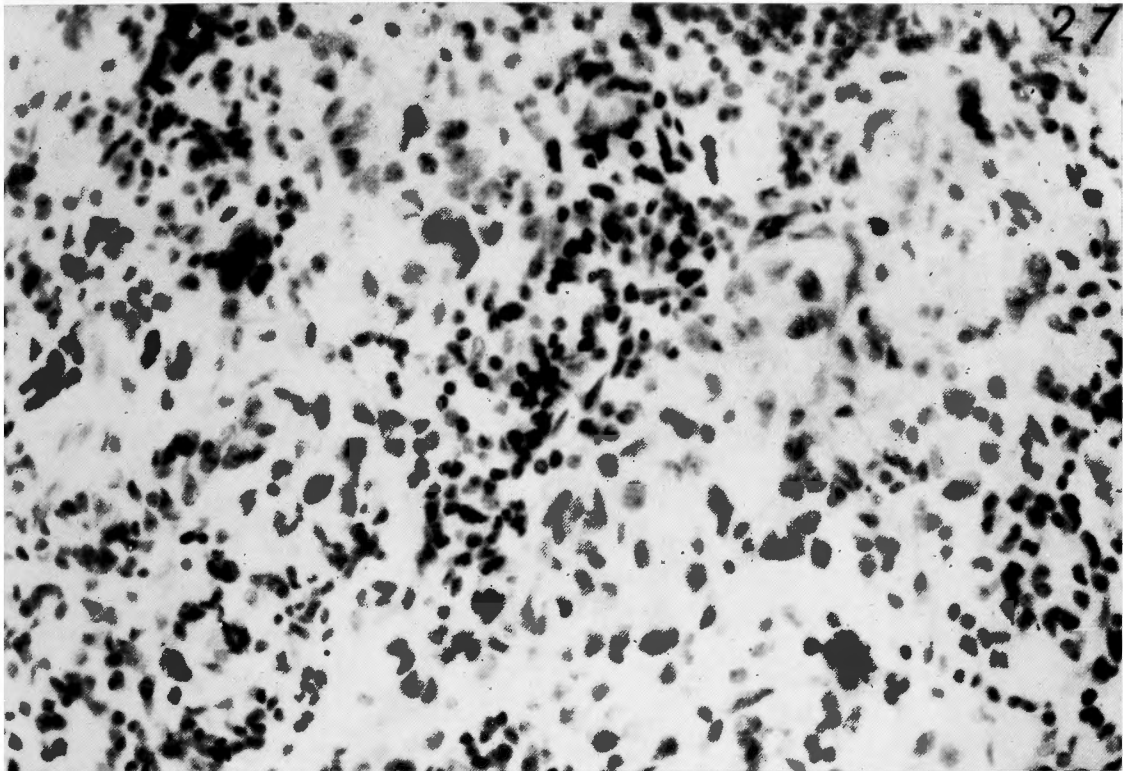
DISCUSSION

Based on the observation that acid phosphatase activity demonstrated by the improved GOMORI's PbS method provided a valuable measure in the gastric exfoliative cytology, further study was made on this method for the better identification of gastric cancer cells.

Though acid phosphatase activity demonstrated by the GOMORI's PbS method is still under debate, it is considered to be useful in the gastric exfoliative cytology because of its prominent activity in the nuclei which is least involved in cell degeneration.

Of considerable interest are the results obtained under various pH of incubating medium. Although acid phosphatase reaction is made usually from pH 4.7 to pH 5.3 of the incubating medium, the observation in the present study was made experimentally under various medium pH adjusted by three kinds of buffer solutions without changing other components. It was revealed that each cell had different enzyme activity at a given pH and had the respective optimum pH. The optimum pH for the enzyme activity of cancer cells ranged from 3.6 to 3.8 and it was also under this pH range that cancer cells showed more remarkable activity than other cells. On the other hand, CHANGUS & DUNLAP⁷⁾ reported that a stable level of the phosphatase activity in the gastric juice of the patient with gastric carcinoma was maintained between pH 3.5 and 8.0 except for a slight rise near pH 4.0 and stated further that there was rapid inactivation below pH 3.5. It was shown in their report that the inactivation of this enzyme was least from pH 3.7 to pH 4.0. They also reported that in most of the patient with pathologically confirmed gastric carcinoma the acid phosphatase level in the gastric content was over 10 units per 100 cc and in most of the patients without gastric carcinoma it was less than 10 units. BENDT & HOFFMANN⁸⁾ reported prominent increase of acid phosphatase in the gastric juice of the patient with an- and subacidity or gastric carcinoma in contrast to that of healthy person. In this respect, the histochemical results of the present study seem to correspond to their results which were due to biochemical analysis of the gastric juice of the patient with gastric carcinoma. It has been already confirmed by

Fig.27 and 28 : The section pretreated with 0.01 M EDTA for 5 minutes and processed principally with GOMORI's PbS method by 8 hour incubation in the medium of pH 3.6 containing magnesium chloride in 0.01 M. Inflammatory cells showing the strongest activity followed by cancer cells. The cellular and nuclear structures are fine. The activity is marked in the nucleus and light in the cytoplasm. $\times 200$ and $\times 1000$.



many biochemical analysis (ABUL-FADL⁹⁾, AKABORI¹⁰⁾ and NOVIKOFF¹¹⁾) that each tissue or cell has its own optimum pH of phosphatase activity. The present histochemical study also revealed that each cell showed various phosphatase activity at a given pH and had respective optimum pH, which was between 3.6 and 3.8 in gastric cancer cells.

The interesting point was that intracellular localization of phosphatase activity varied according to the change of medium pH. Of course, the effect on the intracellular localization of the activity of different buffer solutions must be taken into consideration, but, at any rate, the presence of both nuclear and cytoplasmic activities were recognized histochemically in the present study. PALADE¹²⁾ reported that according to biochemical determination the acid phosphatase was located almost entirely in the cytoplasm, while the GOMORI's histochemical test revealed that the enzyme was predominant in the nucleus. On the other hand, WEINREB¹³⁾ reported that acid phosphatase was predominant in the cytoplasm and very slight in the nucleus according to the GOMORI's technique modified by GOETCH & REYNOLD. At least two kinds of acid phosphatase were demonstrated by SELIGMAN¹⁴⁾ using azo-dye technique, one in the cytoplasm of the prostatic epithelial cell and the other in the nucleus of the prostatic epithelial cell and of stromal cell. Summarizing the above observations, the existence of cytoplasmic and nuclear acid phosphatase activity is doubtless.

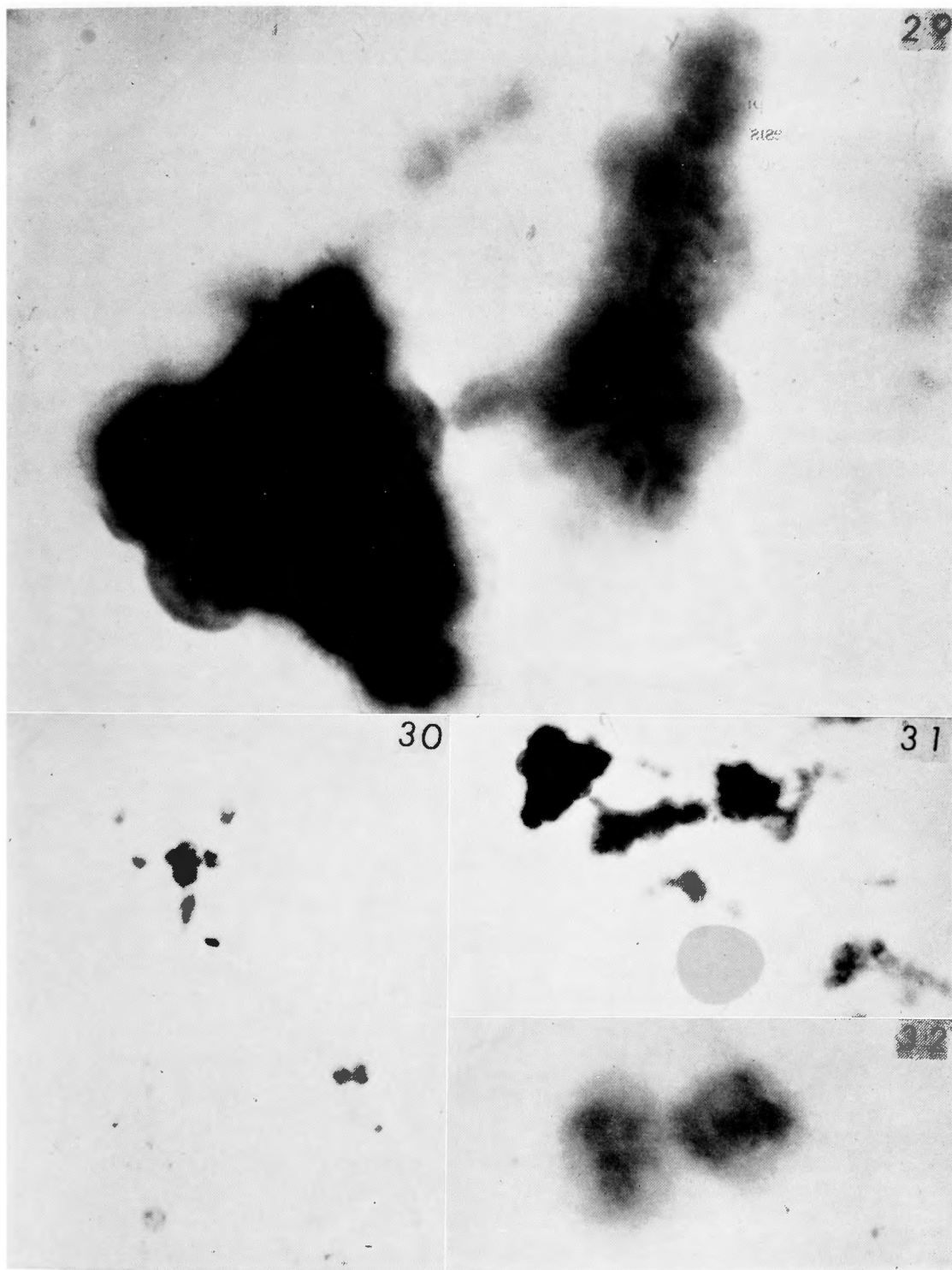
In contradiction to the GOMORI's opinion that magnesium acts as an enzyme activator only on alkaline phosphatase and not on acid phosphatase, it was demonstrated in this study that this ion had influence as an activator on the phosphatase even in acid level such as pH 3.6. And the phosphatase activity was found with all cells over all pH range, though the activity was various in intensity. From these facts, it seems to be unreasonable to draw a definite borderline between acid and alkaline phosphatase exactly at pH 7.0.

Although the staining was remarkably diminished in alkaline site above pH 8.2 due to insufficient solubility of lead salts in the incubating medium, the feature of the staining was similar to that of alkaline phosphatase reaction by the GOMORI's salt method.

Periodic observation of acid phosphatase activity showed that its activity was limited, in a short incubation time, only to nuclear membrane and probable nucleolei, gradually spreaded to the whole nucleus and eventually to the cytoplasm and to the extracellular portion. This observation may suggest that the enzyme diffusion occurs from the nuclei to the cytoplasm or that the grade of the enzyme activity is diminished in the order of probable nucleolei, nuclear membrane, the other portion of nuclei and cytoplasm; therefore, the less the activity, the longer the incubation time required to show enough activity. According to GREEN¹⁵⁾, however, acid phosphatase was demonstrated throughout the cytoplasm and not in the nucleus by means of freeze-dry fixation of Hela cells, and the

Fig.29 and 31 : The acid phosphatase activity is remarkable in cancer cell forming a clump in the cytologic specimen. Nuclear structures are presented slightly in some cancer cells and obscure in others with the marked activity. The author's method. $\times 400$ and $\times 100$.

Fig.30 and 32 : The acid phosphatase activity is marked to moderate in cancer cells in the cytologic specimen with strongly stained areas in the nuclei, probably the nucleolei, and none in others. The author's method. $\times 100$ and $\times 400$.



enzyme activity was increased and spread throughout the cell upon its death. GOMORI also reported that the prominent enzyme activity in the nucleus was due to the diffusion of enzyme and products of its hydrosis which was seen even in the fixed tissue. However, such a hypothesis that the marked nuclear activity is due to the diffusion from the cytoplasm to the nucleus is difficult to explain from the present study.

The study on the specimens pretreated with EDTA revealed that various kinds of ions originally contained in the tissue were necessary for acid phosphatase activity. Especially was this true about calcium and magnesium ion which were indispensable in the enzyme activity, the effect by these ions being various depending on the kinds of cells. Calcium ion activated the enzyme in cancer cells most strongly among others, whereas, magnesium ion activated the enzyme in inflammatory cells more strongly than that in cancer cells, suggesting that there were various kinds of the phosphatase specific to calcium ion or magnesium ion etc, and that cancer cells contain more phosphatase specific to calcium ion than other cells.

In the present study, the fine structure of the nucleus was obtained by adding calcium ion to the incubating medium, while SCHNEIDER¹⁶⁾ using the phase-contrast micrography reported that the morphological feature of the nucleus from the mouse spleen was maintained in the calcium-sucrose medium. BRUNSCHWIG¹⁷⁾, DUNHAM¹⁸⁾ and DELONG¹⁹⁾ reported that in gastric carcinoma and also in the other neoplastic tissues less calcium was found than in the adjacent uninvolved tissues. Therefore, addition of calcium ion to the incubating medium is desirable not only for the activation of the enzyme, but also for the maintenance of morphological integrity of the cells, especially of cancer cells.

GOMORI reported that black or deep brown staining of some structures of high contrast was obtained by the PbS method, while the post-coupling technique resulted in the lighter brown staining, and added that especially good contrasts were obtained with glycerophosphate as substrate. When performing the reaction of acid phosphatase for the better identification of malignant gastric cells, it is desirable to use the PbS method with glycerophosphate as substrate.

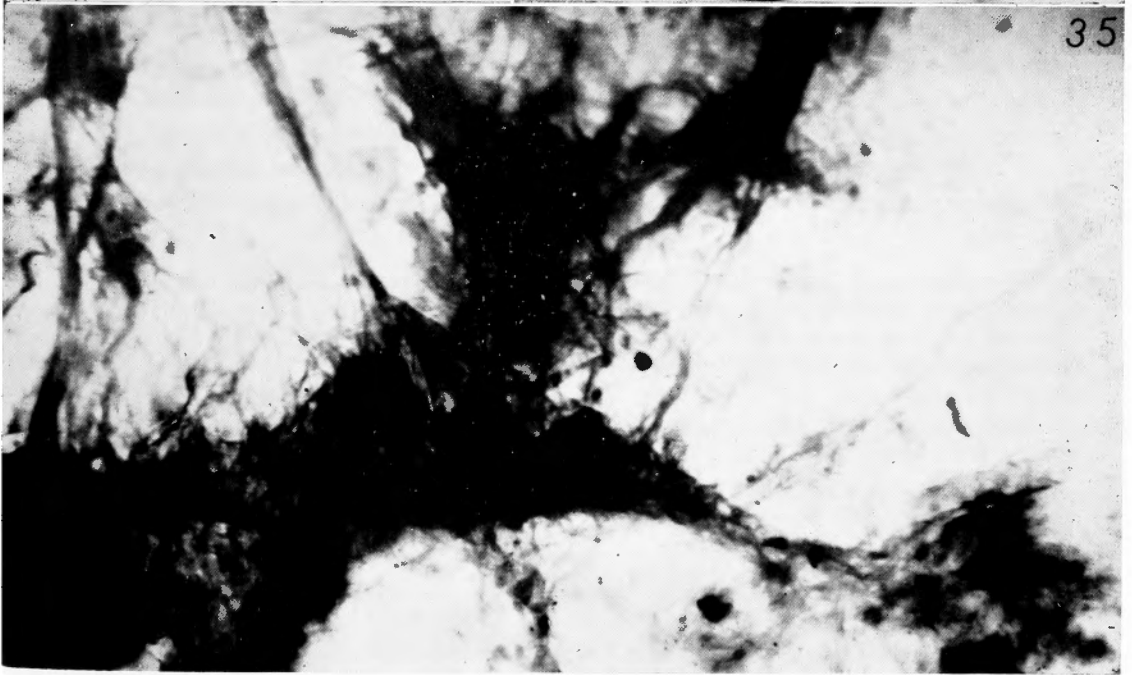
By means of the stain method obtained from this study, the remarkable disparity of acid phosphatase activity was observed between cancer and other cells in the cytologic specimen, which is considered to be of great significance in the gastric exfoliative cytology.

The existence of some exfoliated cancer cells without showing this enzyme activity, however, would be a weak point of this method for its application on the exfoliative cytology. It seems to be inevitable for degenerated cells to be mixed in the cytologic specimen which failed to show the positive enzyme activity. Therefore, the effort should be made to find some method rendering all cancer cells to show the enzyme activity by

Fig.33 : The acid phosphatase activity is marked in cancer cells in the cytologic specimen. The nuclear structures are slightly noted. The author's method. $\times 400$.

Fig.34 : The acid phosphatase activity is remarkable in cancer cells forming a clump in the cytologic specimen. The author's method. $\times 100$.

Fig.35 : The marked activity is noted diffuse in the mucous per se of the cytologic specimen from the patient with gastric carcinoma, whereby cancer cells and inflammatory cells scattered in the mucous also show the prominent activity. The author's method. $\times 100$.



further investigation. At any rate, the proper and rapid maneuver is necessary for the collection and stain process of the cytologic specimen.

What are the reasons creating some difference of staining as to the cellular structure between the cytologic specimen and the section of surgical material? Further studies should be made to find out those reasons and to obtain the finer nuclear structures in all exfoliated cells

In the present study, the specimens were stained without counterstain in order to demonstrate the enzyme activity properly and purely. The achievement of proper counterstain for cell bodies which shows distinct contrast to the enzyme reaction is the problem left for the further study.

SUMMARY

The present study was made on the influence of several factors to acid phosphatase activity by the improved GOMORI's PbS method for the better identification of gastric cancer cells using the resected stomach.

Eight hour incubation was optimal to obtain the fine cellular structure.

The optimum pH for the phosphatase activity in cancer cells was from 3.6 to 3.8, where the activity was more marked in cancer cells than in others.

The enzyme activity of cancer cells was demonstrated most strongly by calcium ion (0.01 M) among all kinds of cells. The fine nuclear structure was obtained by calcium ion.

Thus, the ideal stain method of acid phosphatase activity for the identification of malignant gastric cells is as follows. Specimens pretreated with 0.01 M EDTA for 5 minutes are processed according principally to the GOMORI's PbS method for acid phosphatase reaction, with 8 hour incubation at 37°C in the medium of pH range from 3.6 to 3.8 containing calcium chloride in 0.01 M and glycerophosphate as substrate.

And the cytologic specimen obtained by "abrasive balloon with a sheath" was processed under the above mentioned formula and the acid phosphatase activity was observed moderate to marked in most of cancer cells, but none in some cancer cells and most of other cells.

The author wishes to express sincere gratitude to Dr. RYO INOUE, an instructor of the Surgical Department, for many valuable suggestions and helpful discussions in this investigation. The author's grateful thanks are due to Dr. AKIRA MIZUTANI, an instructor of the Tuberculosis Research Institute of Kyoto University, and also Dr. MASASHI OKUDA, the assistant of the Pathological Division of Kyoto University, for their kind guidances in the course of the work.

The gist of the present study was reported at the 20th General Meeting of the Japanese Cancer Association at Sendai (October 1961).

The work reported in this paper was supported in part by Cancer Research Grant from the Nippon Life Insurance Company, Osaka, Japan.

References

- 1) Yoshida, Y. : The trials in the cytological diagnosis of gastric cancer. (Part III). Arch. Jap. Chir., **31**, 302, 1962.
- 2) Gomori, G. : Microscopic Histochemistry. Univ. Chicago Press, Chicago, 1952.
- 3) Gomori, G. : Histochemical methods for acid phosphatase. J. Histochem. & Cytochem., **4**, 453, 1956.
- 4) Newman, W., Kabat, E. A. & Wolf, A. : Histochemical studies on tissue enzymes. Am. J. Path., **26**,

489, 1950.

- 5) Pearse, A. G. E. : *Histochemistry*. Churchill, London, 1951.
- 6) Lison, L. : *Histochimie et Cytochimie*. Gauthier-Villars, Éditeur, Paris, 1953.
- 7) Changus, G. W. & Dunlap, C. E. : Acid-phosphatase activity in the gastric content of patients with carcinoma of the stomach. *J. Nat. Cancer Inst.*, **10**, 481, 1949.
- 8) Bendt, H. u. Hoffmann, A. : Die Bedeutung der Phosphatasen im Magensaft für die Diagnose des Magencarcinoms. *Acta biol. med. germ.*, **3**, 303, 1959.
- 9) Abul-Fadl, M. A. M. & King, E. J. : Properties of the acid phosphatases of erythrocytes and of the human prostate gland. *Biochem. J.*, **45**, 51, 1949.
- 10)* Akabori, S. : *Method of Study on Enzyme*. Asakura-shoten, Tokyo, 1956.
- 11) Novikoff, A. B. : Distinguishing substrate-specific phosphatases. *J. Histochem. & Cytochem.*, **6**, 251, 1958.
- 12) Palade, G. E. : Intracellular localization of acid phosphatase. *J. Exper. Med.*, **94**, 535, 1951.
- 13) Weinreb, E. L. : Histochemical demonstration of sites of acid phosphatase activity in mitochondria. *Exper. Cell Research*, **8**, 159, 1955.
- 14) Seligman, A. M. & Manheimer, L. H. : A new method for the histochemical demonstration of acid phosphatase. *J. Nat. Cancer Inst.*, **9**, 427, 1948.
- 15) Green, M. H. & Verney, E. L. : A simple method for the study of intracellular acid phosphatase in Hela cells. *J. Histochem. & Cytochem.*, **4**, 106, 1956.
- 16) Schneider, R. M. & Petermann, M. L. : Nuclei from normal and leukemic mouse spleen. *Cancer Research*, **10**, 751, 1950.
- 17) Brunschwig, A., Dunham, L. & Nichols, S. : Potassium and calcium content of gastric carcinoma. *Cancer Research*, **6**, 230, 1946.
- 18) Dunham, L., Nichols, S. & Brunschwig, A. : Potassium and calcium content of carcinomas and papillomas of the colon. *Cancer Research*, **6**, 233, 1946.
- 19) DeLong, R. P., Coman, D. R. & Zeidman, I. : The significance of low calcium and high potassium content in neoplastic tissue. *Cancer*, **3**, 718, 1950.

(* Written in Japanese)

胃癌細胞診の診断適中率向上を目的とする二、三の試み

第四編 胃癌細胞の弁別を目的とした酸性 フォスファターゼ染色法の研究

京都大学医学部外科学教室第二講座（指導：青柳安誠教授）

吉 田 良 行

胃癌細胞をよりよく弁別しようという見地から、手術で切除した胃癌および胃潰瘍組織を用いて、GOMORI氏 acid phosphatase 染色 硫化鉛法（改良法）によって証明される acid phosphatase 活性に影響を与える数種の因子について研究した。

Incubation の時間が長くなると、細胞特に核構造は瀰漫性となり不鮮明となるので、微細な細胞特に核の構造を得るには8時間のincubation が適していると考えられる。

Medium のpHを種々変えてみると、胃癌細胞は全pH領域中、pH3.6~3.8において最大の活性を示し、しかも、このpH領域では胃癌細胞は他の細胞より強い活性を示した。

0.01M の EDT A 液で5分間、処理した組織切片を0.01M の割合に塩化カルシウム、または塩化マグネシウムを含有するmedium に incubate すると、本酵素活性は著明に出現し、細胞特に核の構造は微細、鮮明であつた。特に、カルシウムイオンによつて、全種類の細胞中、癌細胞の本酵素活性が最も強く表出された。

かくして、胃癌細胞をよりよく判別しようとして

acid phosphatase 染色を試みる場合には、次のような条件下に行われるのが理想的である。原則的に GOMORI 氏硫化鉛法に従つて、0.01M の EDTA 液で5分間処理した試料を、基質にはグリセロリン酸を用い、pHを3.6~3.8に調整して、0.01M の割合に塩化カルシウムを含んだ medium に37°C で8時間 incubate するのである。

更に、この組織切片で得た染色条件を以て、*外套管付abrasive balloon* で採取した胃細胞診試料に適用したところ、その結果、大部分の癌細胞が強度或いは中等度の反応を示したのに対して、一部の癌細胞は極めて弱い反応を示すか、または全く反応を示さなかつた。そして、癌以外の他の細胞は稀に極めて弱い反応を示したが、ほとんど全ての細胞は全く反応を示さなかつた。すなわち、癌細胞と非癌細胞の反応の程度に相当の隔差を付けることが出来た。

また、細胞診試料においては、或程度の細胞特に核構造を示す細胞もあつたが核全体が濃染する細胞が可成りあつて、この点、組織切片においては微細鮮明な細胞特に核構造が得られたのと趣を異にする。